Yarrowia lipolytica transformation protocol (Schwartz et al.)

Introduction

Transformation protocol from Cory.

Materials

- Liquid media for O/N of Y. lip strain (YPD)
- Plasmid DNA
- 150 µl 70% PEG (4000)
- 1 ml MilliQ
- **Transformation buffer:**
  - LiAC 0.3 M
  - Tris-HCl (pH 8) 10 mM
  - EDTA (1 mM)
- **SS DNA mix:**
  - ssDNA 8 mg/ml
  - Tris-HCl (pH 8) 10 mM
  - EDTA 1 mM
- **BME mix:**
  - Triacetin 95% (v/v)
  - BME 5% (v/v)

Procedure

Day 1

1. Grow O/N culture of Y. lipolytica strain to be transformed

Day 2

2. Pellet cells (250 ul if YPD, 1 ml if SD-X) and discard supernatant (all spins at 5000x RPM for 2 min)
3. Wash cells by resuspending and mixing via pipette with 250 ul transformation buffer
4. Pellet cells and discard supernatant
5. Resuspend in 100 ul transformation buffer
6. Add the following (in order)
   a. 3 ul SS DNA mix
   b. 0.5-5 ug plasmid DNA in water
   c. 15 ul BME mix

7. Ensure well mixed with pipette (ensure BME mix is not settled)
8. Incubate 30 minutes at RT
9. Add 150 ul 70% PEG (4000)
10. Ensure well mixed with pipette
11. Incubate 30 minutes at RT
12. Heat shock – 15 minutes at 37C
13. Add 1 ml H20, mix via pipetting
14. Pellet cells and discard supernatant
15. Resuspend cells in desired volume MQ and plate or use to inoculate liquid culture (or both)